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FORMULATION AND EVALUATION OF CAPSULE FOR THE TREATMENT OF ASPERGILLOSIS

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ABSTRACT:

Aspergillosis, a severe fungal infection caused primarily by the genus Aspergillus, poses significant challenges in clinical management due to its resistance to conventional antifungal therapies. In recent years, there has been growing interest in exploring alternative treatment options, including the development of novel formulations utilizing natural compounds with antifungal properties. This study aims to formulate and evaluate a capsule for the treatment of aspergillosis, leveraging advancements in pharmacology and biotechnology.Drawing inspiration from traditional medicine systems like Ayurveda, which emphasize the therapeutic potential of herbal remedies, this research focuses on harnessing the medicinal properties of select botanical extracts known for their antifungal activities. Key among these is azadirachtin indica , a plant species renowned for its diverse pharmacological properties, including antimicrobial effects. In this study, various extraction methods will be employed to obtain bioactive compounds from azadirachtin indica and other potent botanical sources. These compounds will then be incorporated into capsule formulations using pharmaceutical excipients to ensure stability, bioavailability, and efficacy.The formulated capsules will undergo comprehensive evaluation to assess their antifungal activity against Aspergillus strains commonly implicated in human infections. In vitro studies will investigate the capsules' inhibitory effects on fungal growth and viability, while in vivo experiments using animal models will provide insights into their therapeutic efficacy and safety profile. Overall, this research endeavors to contribute to the development of effective and affordable treatment options for aspergillosis, addressing the urgent need for novel antifungal therapies in clinical practice. By combining insights from traditional medicine with modern pharmaceutical science, the formulated capsules hold promise in combating this life-threatening fungal infection and improving patient outcomes.

Objective: The objective of this study was to formulate and evaluate capsule utilizing azadirachitin indica as a key ingredient, aiming to harness its potential therapeutic benefits for aspergillosis conditions.

Purpose: The purpose of this research was to develop a capsule that offers anti-aspergillosic effect , utilizing azadirachitin indica as a natural and potentially effective active ingredient. This capsule could serve as a safer and more accessible alternative for managing aspergillosis condition.

Result: The formulated capsule demonstrated promising activity against aspergillosis in both in vitro assays and in vivo experiments, exhibiting inhibition of aspergillosis markers and reducing infaction of aspergillosis in animal models. Additionally, the capsule showed favorable physical characteristics, stability, and safety profiles, making it suitable for oral application.

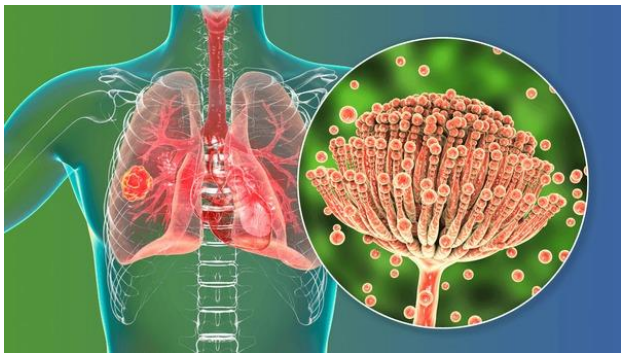
Conclusions: This study concludes that the developed capsule incorporating capsule holds potential as a novel therapeutic option for managing aspergillosis conditions. Further research and clinical trials are warranted to explore its efficacy, safety, and clinical applications in greater depth.

Keywords: Aspergillosis, Herbal capsule, Antifungal activity, Formulation, Evaluation, Herbal medicine, Therapeutic efficacy, Drug resistance, Pharmacological properties and Bioactive compounds

INTRODUCTION:

Aspergillosis is a group of diseases caused by a type of fungus called Aspergillus. It can range from mild allergic reactions to severe lung infections. There are different types of aspergillosis, including allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA), invasive aspergillosis (IA), and aspergilloma (fungus ball).Symptoms vary depending on the type of aspergillosis and the organs affected but may include cough, wheezing, shortness of breath, combination of imaging tests, such as chest X-rays or CT scans, and laboratory tests, including

sputum cultures and blood tests. Treatment depends on the severity and type of infection but often includes antifungal medications. In severe cases, surgery may be necessary to remove infected tissue.Prevention strategies include avoiding exposure to environments with high levels of mold, especially for individuals with weakened immune systems, and taking precautions such as wearing masks and gloves when working with moldy materials. The significance of this research endeavor extends beyond the confines of academia, with profound implications for public health and clinical practice. By harnessing innovative formulation strategies and rigorous evaluation methodologies, this project aspires to catalyze the development of next-generation therapeutic interventions for Aspergillosis, thereby ameliorating patient outcomes, alleviating healthcare burdens, and advancing the frontier of medical science in combating this formidable fungal infection. Through a concerted effort encompassing basic research, translational studies, and clinical trials, this project seeks to pave the way towards a paradigm shift in Aspergillosis therapeutics, heralding a new era of efficacy, safety, and patient-centered care.



(1) Fig of Infection (2)

Aspergillosis : Aspergillus is a group of fungi. Certain types can lead to respiratory infections, particularly in those with weakened immune systems. It's present in soil, decaying matter, and indoors, posing health risks.

History of Aspergillosis

- Early Observations (18th Century): Physicians in the 18th century documented cases of pulmonary aspergillosis, describing symptoms such as coughing, fever, and difficulty breathing in individuals exposed to moldy environments. However, the connection between these symptoms and fungal infection was not fully understood at the time.
- Identification of Aspergillus (19th Century): In the late 19th century, Italian physician Giovanni Battista Amici identified and named the fungus Aspergillus, derived from its resemblance to the shape of an aspergillum, a liturgical implement used for sprinkling holy water. This discovery laid the foundation for understanding the etiology of aspergillosis.
- Advancements in Microbiology (20th Century): Throughout the 20th century, advancements in microbiology and medical technology led to a better understanding of Aspergillus species and their role in human disease. Researchers identified various \
- Classification and Clinical Manifestations: Aspergillosis was classified into different forms based on clinical manifestations, including allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA), invasive pulmonary aspergillosis (IPA), and others. Each form of aspergillosis has distinct symptoms, risk factors, and treatment approaches

Types of Aspergillosis :

Aspergillosis can be categorized into different types based on various factors such as duration, cause, and location. Here are some common types of Aspergillosis :

Type of Inflammation	Description	Treatment
Allergic Bronchopulmonary Aspergillosis (ABPA	**Allergic reaction to Aspergillus spores, primarily affecting individuals with asthma or cystic fibrosis.**	**Corticosteroids (e.g., prednisone), antifungal medications (e.g., itraconazole), and managing underlying asthma or cystic fibrosis.**

Chronic Pulmonary Aspergillosis (CPA	**Long-term infection causing cavities in the lungs, which may result in fungal balls (aspergillomas).**	**Antifungal medications (e.g., voriconazole or itraconazole), sometimes surgical removal of aspergillomas, and addressing any underlying lung conditions.**
3) Invasive Aspergillosis (IA)	**Serious infection that can spread to other parts of the body, typically seen in immunocompromised individuals.**	**High-dose intravenous antifungal medications (e.g., voriconazole, isavuconazole, or liposomal amphotericin B), and supportive care for organ function.**
4) Aspergilloma	**Fungal ball that grows in pre-existing lung cavities formed by conditions like tuberculosis or sarcoidosis**	**Observation if asymptomatic, surgical removal if symptomatic, and antifungal medications in some cases.**
5)Cutaneous Aspergillosis	**Infection of the skin, which can occur as a primary infection or secondary to dissemination from an invasive aspergillosis.**	**Antifungal medications (e.g., voriconazole or itraconazole), surgical debridement of affected areas, and addressing underlying immunosuppression.**
6) Allergic Aspergillus Sinusitis	**Allergic reaction causing inflammation and blockage in the sinuses..**	**Corticosteroids, antifungal medications (e.g., itraconazole), and sometimes surgery to clear sinus obstructions.**

7) Aspergillus Otomycosis	**Fungal infection of the external ear canal..**	**Topical antifungal treatments (e.g., clotrimazole drops), cleaning of the ear canal, and managing underlying conditions predisposing to infection.**

Causes of Aspergillosis :

1. Exposure to Aspergillus Spores: Inhaling spores from the Aspergillus mold is the direct cause. These spores are commonly found in soil, decaying leaves, compost, and dust.
2. Weakened Immune System: Individuals with weakened immune systems are more susceptible. This includes people undergoing chemotherapy, organ or stem cell transplants, or those with conditions like HIV/AIDS.
3. Lung Conditions: People with existing lung conditions, such as tuberculosis, chronic obstructive pulmonary disease (COPD), or cystic fibrosis, are at higher risk.
4. Corticosteroid Use: Long-term use of corticosteroids can suppress the immune system, increasing the risk of aspergillosis.
5. Hospital Environments: Hospitals can be a source of Aspergillus exposure, especially during construction or renovation when spores can be released into the air.
6. Environmental Exposure: Activities that stir up dust and spores, such as gardening, farming, or working with compost, can increase the risk of inhaling Aspergillus spores.

Capsule :

Hard gelatin capsules are solid dosage forms consisting of two parts: a body and a cap, which fit together to enclose the medication. They are commonly used in the pharmaceutical industry to deliver drugs orally.Composition:Gelatin: Derived from collagen, typically sourced from bovine or porcine skin and bones.Water: Constitutes 12-16% of the capsule weight.Colorants and Opacifiers: Such as titanium dioxide, to provide color and opacity.Preservatives: Sometimes added to prevent microbial growth.

Key features of capsule include:

- Composition:Gelatin: Made from collagen, primarily sourced from bovine or porcine skin and bones.Water: Contains 12-16% water, essential for maintaining flexibility.
- Additives: May include colorants, opacifiers (e.g., titanium dioxide), and preservatives.
- Structure:Two-Piece
- Design: Consists of a body and a cap that fit together to enclose the medication.
- Variety of Sizes: Available in different sizes to accommodate various dosages and formulations.
- Versatility:Filling Options: Suitable for powders, granules, pellets, tablets, and certain liquids.
- Masking Capabilities: Can mask unpleasant tastes and odors of encapsulated drugs.
- Bioavailability:
- Rapid Disintegration: Quickly dissolves in the stomach, leading to rapid drug release and absorption.
- Manufacturing Process:Dipping and Drying: Gelatin forms are dipped, dried, stripped, trimmed, and joined to form the capsule.
- Quality Control: Ensures uniformity in size, weight, and dissolution properties.6. Storage Conditions:Temperature: Best stored at 15-25°C.
- Humidity: Optimal relative humidity is 35-65% to prevent brittleness or excessive softening.
- Safety and Compliance:Regulatory Standards: Produced in compliance with pharmaceutical standards to ensure safety and efficacy.Tamper-Evident: Can be designed with features that indicate if the capsule has been tampered with.
- Applications:
- Pharmaceuticals: Encapsulation of prescription and over-the-counter medications.
- Nutraceuticals: Used for vitamins, minerals, and dietary supplements.

- Herbal Products: Suitable for herbal extracts and powders.
- Consumer Preferences:Ease of Swallowing: Smooth texture and shape facilitate easy swallowing.Vegetarian Options: Alternative materials like hydroxypropyl methylcellulose (HPMC) are available for vegetarians and vegans.

Types of Capsule :

Capsules are a common form of oral medication delivery, and they come in various types based on their materials, release mechanisms, and intended use. Here are the main types of capsules:

1. Hard Gelatin Capsules :
- Made from gelatin derived from animal collagen.
- Consist of two pieces: a body and a cap.
- Used for solid or powdered medications.

2. Soft Gelatin Capsules (Softgels) :
- Made from a more flexible gelatin, often with added glycerin or sorbitol.
- Usually one-piece and hermetically sealed.
- Ideal for oils and liquids.

3. Vegetarian Capsules :
- Made from plant-derived materials such as hydroxypropyl methylcellulose (HPMC) or pullulan.
- Suitable for vegetarians and vegans.
- Available in both hard and soft forms.

4. Enteric-Coated Capsules :
- Designed to withstand the acidic environment of the stomach and dissolve in the more neutral or alkaline environment of the intestines.
- Used to protect sensitive ingredients or to target intestinal absorption.

5. Extended-Release Capsules :
- Formulated to release the active ingredient gradually over an extended period.
- Helps maintain steady drug levels in the bloodstream.

6. Liquid-Filled Hard Capsules (LFHC):
- Hard capsules filled with a liquid, often with a band to seal them.
- Useful for liquid or semi-solid formulations that need precise dosing.

7. Modified-Release Capsules :
- Includes various subtypes like delayed-release, sustained-release, and controlled-release.
- Designed to modify the timing or location of drug release.

8. Pulmonary Capsules :
- Specifically designed for inhalation rather than oral consumption.
- Used with inhalers to deliver medication directly to the lungs.

9. Gastro-Resistant Capsules :
- Similar to enteric-coated, but specifically formulated to resist gastric fluids.
- Ensures that the medication reaches the intestine intact.

10. Sublingual and Buccal Capsules :
- Designed to dissolve under the tongue (sublingual) or between the gums and cheek (buccal).
- Allows for rapid absorption into the bloodstream through the mucous membranes.

Types of Granules

These various capsule types allow for a wide range of applications and enhance the effectiveness of the medication by tailoring the release profile and protecting the active ingredients.

Granules are small, dry particles or pellets used in pharmaceuticals, food, and other industries. In pharmaceuticals, they are often used for oral medication delivery. Here are the main types of granules:

1. Effervescent Granules :
- Contain an acid (like citric acid) and a base (like sodium bicarbonate) that react when in contact with water to release carbon dioxide, creating a fizzy solution.
- Used for medications that are intended to be dissolved in water before consumption.

2. Sustained-Release Granules :
- Formulated to release the active ingredient slowly over time.
- Helps maintain a consistent drug level in the bloodstream over an extended period.

3. Enteric-Coated Granules:

- Coated to resist the acidic environment of the stomach and dissolve in the more neutral or alkaline environment of the intestines.
- Used to protect drugs that are sensitive to stomach acid or to target intestinal absorption.

4. Effervescent Granules :
   - Designed to dissolve quickly in water, releasing carbon dioxide and creating a fizzy solution.
   - Commonly used for vitamins and minerals.
5. Dispersible Granules :
   - Formulated to be dispersed in water or other liquids, creating a suspension for easy swallowing.
   - Often used for pediatric or geriatric patients who have difficulty swallowing tablets or capsules.
6. Immediate-Release Granules :
   - Designed to dissolve quickly after administration, allowing for rapid onset of action.
   - Typically used for pain relievers and other medications that require fast absorption.
7. Gastro-Resistant Granules :
   - Similar to enteric-coated, but specifically formulated to resist breakdown in the stomach and dissolve in the intestines.
   - Ensures the medication is released where it is needed.
8. Chewable Granules :
   - Formulated to be chewed and swallowed without the need for water.
   - Often flavored to improve palatability, making them suitable for children.
9. Modified-Release Granule :
   - Includes various types such as delayed-release, extended-release, and controlled-release granules.
   - Designed to modify the timing or location of drug release.
10. Orally Disintegrating Granules (ODGs) :
    - Designed to disintegrate quickly in the mouth without the need for water.
    - Useful for patients who have difficulty swallowing tablets or capsules.
11. Nutritional Granules :
    - Used to deliver vitamins, minerals, and other nutritional supplements.
    - Often formulated to dissolve in water or other beverages.

These types of granules allow for flexible dosing, improved stability, and targeted drug delivery, enhancing the effectiveness and convenience of medications and supplements.

- **Preparation Methods of capsule and Granules**

Preparation Method	Description
Direct Filling	**Fill capsules directly with powdered or granular form of the active ingredient and excipients..**
Manual Filling	**Fill capsules manually using small-scale filling trays or machines, often for small batches.**
Semi-Automatic Filling	**Use semi-automatic machines to fill capsules, which involve some manual handling but automate filling.**
Automatic Filling	**Employ fully automated machines for high-volume production, where capsules are filled and sealed in bulk.**
Liquid or Semi-solid Filling	**Fill capsules with liquid or semi-solid formulations, often followed by sealing to prevent leakage.**
Encapsulation Of Pellets	**Fill capsules with preformed pellets containing the active ingredient for controlled release.**
Sealing\Banding	**Apply a band or seal to the capsule joint to prevent tampering and enhance stability.**
Hot-Melt Filling	**Fill capsules with a melted mass that solidifies upon cooling, typically used for lipid-based formulations.**

Advantages of Capsule :

- Hard gelatin capsules offer several advantages:
- Versatility: They can encapsulate a wide range of substances, including powders, granules, semi-solids, and even certain liquids.
- Bioavailability: They can improve the bioavailability of the encapsulated drug, ensuring better absorption and effectiveness.
- Ease of Use: They are easy to swallow, making them a preferred choice for many patients.
- protection: They provide a good barrier against light and air, helping to protect sensitive ingredients from degradation.
- Customizable: They can be manufactured in various sizes and colors, allowing for brand differentiation and ease of identification.
- Reduced Processing Cost : Compared to some other forms, hard gelatin capsules can be filled and processed relatively easily and

quickly.

- Improved Patient Compliance : Their smooth texture and lack of taste help improve patient compliance compared to tablets or other forms of medication.
- Tamper Evident: They can be made tamper-evident, providing added security against contamination or tampering.
- These benefits make hard gelatin capsules a popular choice in pharmaceutical and nutraceutical applications

(A) PLANT PROFILE:

Fig. of Plant Neem

1. **Common Names:**
2. Neem
3. Indian Lilac
4. Nimtree
5. **Scientific Classification:**
6. Kingdom: Plantae
7. Order: Sapindales
8. Family: Meliaceae
9. Genus: Azadirachta
10. Species: A. indica
11. **Description:** Azadirachta indica, commonly known as neem, is a fast-growing, evergreen tree native to the Indian subcontinent. It is widely cultivated in tropical and subtropical regions.
12. **Physical Characteristics:**
13. **Height:** Can grow up to 15-30 meters (49-98 feet) tall.
14. **Trunk**: Straight, with a diameter of 1-2.5 meters (3-8 feet).Bark: Hard and fissured, greyish-brown in color.
15. **Leaves:** Pinnate with 20-30 cm (8-12 inches) long leaves, each with 20-31 medium to dark green leaflets.
16. **Flowers**: Small, white to yellowish, fragrant, arranged in drooping panicles.
17. **Fruit**: Smooth, olive-like drupe, 1.4-2.8 cm long, yellow when ripe, containing a single seed.
18. **Habitat and Distribution:**
19. **Native Range**: India, Nepal, Pakistan, Bangladesh, and Sri Lanka.
20. **Introduced Range**: Widely planted in Africa, the Americas, Australia, and Southeast Asia.
21. **Preferred Climate**: Tropical and subtropical regions, thriving in temperatures between 21-32°C (70-90°F) and annual rainfall of 400-1200 mm.
22. **Soil Requirements:** Grows in a variety of soils, including clay, saline, and alkaline soils. Prefers well-drained, sandy loam.
23. **Uses and Benefits:**
24. **Medicinal Uses:**Antibacterial, Antifungal, and Antiviral.
25. **Properties:** Effective against various infections and skin conditions**.**
26. **Anti-inflammatory and Antipyretic**: Used to treat inflammation and fever.
27. **Digestive Health:** Helps in treating gastrointestinal problems**.**
28. **Diabetes Management**: Used in traditional medicine to help control blood sugar levels.
29. **Agricultural Uses:**
30. **Pesticide:** Neem oil and extracts act as natural insect repellents and pesticides.
31. **Soil Improvement:** Neem cake, a byproduct of oil extraction, is used as a natural fertilizer and soil conditioner**.**
32. **Cosmetic Uses:**
33. **Skincare:** Neem extracts are used in soaps, lotions, and creams for treating acne and other skin issues.
34. **Haircare:** Neem oil is used to treat dandruff and promote healthy hair growth.
35. **Environmental Uses:**
36. **Afforestation:** Planted to combat desertification and improve soil fertility.
37. **Carbon Sequestration:** Acts as a carbon sink, helping in reducing atmospheric CO2 levels.
38. **Active Compounds:**
39. **Azadirachtin**: The primary active compound responsible for neem's pesticidal properties.
40. **Nimbin, Nimbidin, and Nimbidol:** Compounds contributing to its medicinal properties.
41. **Sialin and Quercetin:** Known for their anti-inflammatory and antioxidant activities.
42. **Propagation and Cultivation:**Seed Propagation: Fresh seeds germinate readily, but viability decreases quickly.
43. **Vegetative Propagation:** Cuttings and root suckers can be used**.**
44. **Planting:** Best planted at the onset of the rainy season. Requires spacing of 6-8 meters between trees.
45. Challenges:

46. **Seed Viability:** Seeds lose viability quickly, requiring immediate planting after harvesting.
47. **Pests and Diseases:** Susceptible to scale insects and fungal infections, although relatively resilient.

(4) Fig : Plant Profile

(B) DRUG PROFILE:

Chemical Information:

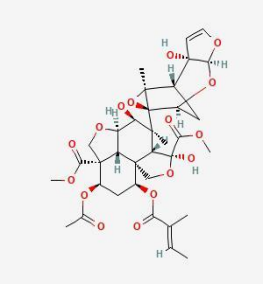
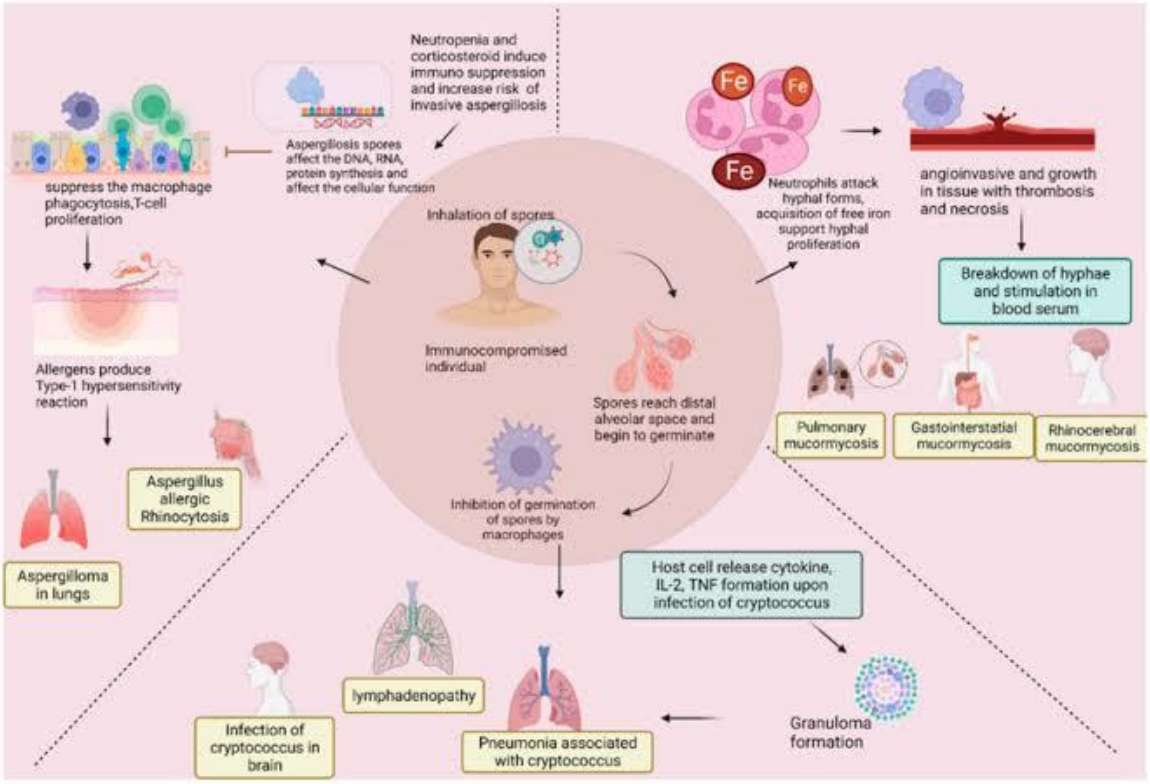


Fig of Azadirachitin

IUPAC//Name:(1R,2S,4S,8R,10S,11S,14R,15S,17S,18R,21R,22R,24S)-2,17,21,22,23,24 Hexahydroxy3,8,11,14,20-pentamethyl-25-oxo-19,26 dioxaheptacyclo[19.6.1.0^{2,18}.0^{4,15}.0^{5,10}.0^{8,10}.0^{14,24}]hexacosa-5-en-16-yl (E)-2-methylbut-2 enoate
Molecular Formula: C35H44O16
Molecular Weight: 720.71 g/mol
Source: Extracted from seeds and other parts of the neem tree (Azadirachta indica).
Mechanism of Action:
Insecticidal Properties: Azadirachtin interferes with the hormonal system of insects, disrupting growth and reproduction. It acts as an antifeedant, repellent, and growth regulator, inhibiting molting and causing sterility in some insects.
Mode of Action: Blocks the release of molting hormones (ecdysteroids) necessary for insect development, leading to death or abnormal growth.
Pharmacological Properties:
Antifeedant: Discourages feeding by making treated plants unpalatable to insects.
Growth Inhibitor: Interferes with insect development stages, preventing larvae from maturing into adults.
Reproductive Inhibitor: Reduces insect fertility and egg viability.
Uses and Applications:Agricultural
Applications:Biopesticide: Used to control a wide range of agricultural pests including aphids, beetles, caterpillars, mites, and nematodes**.**
Organic Farming: Preferred in organic farming due to its natural origin and low environmental impact.
Integrated Pest Management (IPM): Often used in conjunction with other pest control methods to minimize chemical pesticide use.
Medicinal Applications:
Antimicrobial: Exhibits antibacterial, antifungal, and antiviral activities. Used in traditional medicine for treating infections and skin disorders**.**
Anti-inflammatory: Potential use in formulations for reducing inflammation.
Formulations and Dosage:
Formulations: Available in various forms including emulsifiable concentrates, wettable powders, granules, and ready-to-use sprays.
Application Rates: Typically used at concentrations ranging from 0.1% to 1% depending on the type of crop and target pest.
Safety and Toxicology:
Human Safety: Generally considered safe for humans. No significant toxicity reported at typical usage levels. However, prolonged or high-dose exposure can cause mild irritation to skin and mucous membranes.
Environmental Impact: Biodegradable and has a low impact on non-target organisms such as bees, birds, and mammals. However, care should be taken to minimize runoff into water bodies as it can affect aquatic life.
Challenges and Limitations:
Photodegradation: Degrades rapidly when exposed to sunlight, reducing its effectiveness. Formulations may include stabilizers to prolong activity.
Short Residual Activity: Requires frequent applications to maintain efficacy.
Variable Efficacy: Effectiveness can vary based on pest species and environmental conditions.
Storage and Handling:
Storage: Should be stored in a cool, dry place away from direct sunlight to prevent degradation.
Handling: Use appropriate protective equipment such as gloves and masks when handling concentrated forms.



(6) Fig : Mechanism of Aspergillosis

Entry and Initial Infection: Aspergillus spores, or conidia, are ubiquitous in the environment and are typically inhaled into the respiratory tract. In immunocompetent individuals, these spores are usually cleared by the innate immune system. However, in immunocompromised individuals, such as those with neutropenia, hematologic malignancies, or undergoing immunosuppressive therapy, the spores can evade the immune defenses**.**
Colonization and Germination: Once inhaled, the spores can adhere to the epithelial cells in the alveoli and begin to germinate into hyphae. This process is facilitated by the weakened immune response in susceptible hosts.
Tissue Invasion: The hyphae penetrate the epithelial cells and invade the surrounding tissue. Aspergillus produces a variety of enzymes, including proteases, phospholipases, and hemolysins, that degrade host tissue and facilitate invasion.
Immune Evasion and Inflammatory Response: The fungus can evade the host immune response by producing substances like gliotoxin, which suppresses macrophage and neutrophil function. The immune system's attempt to fight the infection leads to an inflammatory response, causing tissue damage and further aiding the spread of the fungus.
Dissemination: In severe cases, the fungus can invade blood vessels (angioinvasion), leading to thrombosis, infarction, and dissemination of the infection to other organs, such as the brain, kidneys, liver, and skin.The specific manifestations of aspergillosis depend on the form of the disease, which can be categorized .
Allergic Bronchopulmonary Aspergillosis (ABPA): A hypersensitivity reaction to Aspergillus antigens leading to airway inflammation and bronchiectasis.Chronic Pulmonary Aspergillosis: Characterized by the formation of aspergillomas (fungal balls) in pre-existing lung cavities or chronic necrotizing pulmonary aspergillosis, a slowly progressive infection causing cavitary lesions.
Invasive Aspergillosis: A severe, rapidly progressing infection that typically affects immunocompromised individuals, leading to high mortality if not promptly treated.

Phytochemical Analysis:

Sample Preparation:

Extraction: Plant material is usually dried, powdered, and subjected to solvent extraction using solvents like ethanol, methanol, acetone, or water to obtain the phytochemical-rich extract. Techniques like Soxhlet extraction, maceration, or ultrasonic extraction can be used.
Preliminary Phytochemical Screening: Qualitative Tests: Basic qualitative tests are performed to identify the presence of different classes of

phytochemicals such as alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phenolic compounds. These tests involve colorimetric reactions specific to each class of compounds.

Alkaloids: Dragendorff's test, Mayer's test.Flavonoids: Shinoda test, alkaline reagent test.Tannins: Ferric chloride test, gelatin test.

Saponins: Froth test, foam test.Terpenoids: Salkowski test.Glycosides: Keller-Kiliani test for cardiac glycosides**.**

Phenolics: Ferric chloride test.

Quantitative Analysis: Spectrophotometric Methods: Used for quantifying specific classes of compounds. For example, the Folin-Ciocalteu method is used for total phenolic content, and the aluminum chloride colorimetric method is used for total flavonoid content.

Chromatographic Techniques: High-performance liquid chromatography (HPLC), gas chromatography (GC), and thin-layer chromatography (TLC) are employed for separating, identifying, and quantifying individual phytochemicals. HPLC is particularly useful for quantifying known antifungal compounds.

Mass Spectrometry (MS): Coupled with chromatography (GC-MS, LC-MS) for precise identification and quantification of phytochemicals.

Nuclear Magnetic Resonance (NMR) Spectroscopy: For structural elucidation of isolated compounds.

Antifungal Activity Assays:

In Vitro Testing: The plant extract and isolated compounds are tested against Aspergillus species using methods like the agar diffusion method (disc or well diffusion), broth dilution method, or microdilution method to determine minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC).Synergistic Studies: Sometimes, the combined effect of phytochemicals and conventional antifungal drugs is evaluated to determine potential synergistic effects.

Characterization of Active Compounds:

Isolation and Purification: Bioactive compounds are isolated using techniques such as column chromatography and further purified by preparative HPLC**.**

Structural Identification: Techniques like MS, NMR, and infrared spectroscopy (IR) are used to determine the structure of active compounds**.**

EXTRACTION OF AZADIRACHITIN : (Macearation Extraction Method)

1. **Preparation of Neem Powder:**Ensure the neem seeds or leaves are dried and ground into a fine powder. This increases the surface area for better extraction.
2. **Solvent Selection:**Ethanol or methanol are commonly used for their efficiency in extracting azadirachtin. Ethanol is often preferred due to its lower toxicity.
3. **Maceration Setup:**Weigh an appropriate amount of neem powder (e.g., 100 grams).Place the neem powder in a glass jar or flask.Add the solvent to the jar at a ratio of approximately 1:10 (powder to solvent, by weight). For instance, if using 100 grams of neem powder, add 1 liter of ethanol.
4. **Maceration Process:**Seal the jar or cover the flask to prevent solvent evaporation.Let the mixture stand at room temperature, stirring occasionally with a stirring rod. Stirring helps to ensure all the powder is adequately exposed to the solvent.Allow the mixture to macerate for 48-72 hours. The extended time allows for thorough extraction of azadirachtin and other compounds.
5. **Filtration:**After maceration, filter the mixture using filter paper or a suitable filtration setup to separate the neem powder residues from the solvent extract. Collect the filtrate in a clean container.
6. **Solvent Removal:**Use a rotary evaporator to remove the solvent from the filtrate under reduced pressure. This helps to concentrate the azadirachtin extract without applying excessive heat, which could degrade the compound.Set the rotary evaporator to an appropriate temperature (around 40-50°C) and apply vacuum to evaporate the solvent.Continue until most of the solvent is removed, leaving behind a concentrated azadirachtin extract.
7. **Drying and Storage:**The concentrated extract can be further dried under a gentle stream of nitrogen or in a vacuum oven at low temperature to remove any residual solvent.Store the dried azadirachtin extract in an airtight container, preferably in a dark and cool place to preserve its stability.

Fig. Of macearatiion Extraction

Identification And Screening of Phytochemical constituents:

Shinoda Test: Mix the extract with magnesium turnings and hydrochloric acid. A red or pink color indicates flavonoids.
Alkaline Reagent Test: Add a few drops of sodium hydroxide to the extract. A yellow color that disappears upon adding dilute acid suggests flavonoids.

Fig of Phytochemical Tests

(A) FORMULATION OF CAPSULE :

Material:

1. Materials and Equipment
2. Active Ingredient: Azadirachtin extract (converted into granules)
3. Excipients: Fillers, binders, disintegrants, lubricants (e.g., lactose, microcrystalline cellulose, starch, magnesium stearate)
4. Solvent: Ethanol or water for granulationCapsule Shells: Hard gelatin or HPMC (hydroxypropyl methylcellulose) High-shear granulator, fluid bed granulator, or simple mortar and pestle for small-scale production.

Drying Equipment: Tray dryer, fluid bed dryer
Sieving Equipment: Sieve or mesh
Encapsulation Machine: Manual capsule filler or automatic encapsulation machine
Mixing Equipment: V-blender, ribbon blender, or planetary mixerProcess

5. **Steps1**. **Preparation of Granules Mixing:** Mix the azadirachtin extract with appropriate excipients (e.g., lactose, microcrystalline cellulose) to ensure even distribution of the active ingredient.

Granulation
Wet Granulation
Solution Preparation: Dissolve a binder (e.g., PVP, starch paste) in a suitable solvent (water or ethanol).Wet **Massing**: Add the binder solution to the powder mix while stirring to form a wet mass.
Granulation: Pass the wet mass through a sieve to form granules.
Dry Granulation: If a wet binder is not suitable, use a dry binder and compress the powder mix into slugs or sheets, then break them down into granules.
Drying: Dry the granules using a tray dryer or fluid bed dryer to remove moisture.
Sieving: Sieve the dried granules to obtain uniform size.

6. **2. Blending with ExcipientsMixing**: Blend the dried granules with additional excipients such as disintegrants (e.g., starch, croscarmellose sodium), lubricants (e.g., magnesium stearate), and glidants (e.g., colloidal silicon dioxide) to improve flow properties and ensure proper capsule filling.
7. **3. EncapsulationCapsule Filling:Manual Filling**: For small-scale production, use a manual capsule filler. Fill the capsule body with the granule blend and cap it.Automatic Filling: For large-scale production, use an automatic encapsulation machine. Adjust the machine settings to ensure consistent fill weight and proper sealing of the capsules.Capsule Sizes: Choose the appropriate capsule size based on the volume and density of the granules. Common sizes range from 000 to 5, with 0 and 1 being typical for most formulations.
8. **4. Quality ControlUniformity of Weight:** Check the weight of filled capsules to ensure uniformity.Disintegration Test: Test the

capsules for disintegration time to ensure they release the granules appropriately.

9. **Assay of Active Ingredient**: Perform an assay to determine the content of azadirachtin in the capsules to ensure each capsule contains the correct dose.Dissolution Test: Conduct a dissolution test to study the release profile of azadirachtin from the capsules.

Formulation Table:

Ingredients	Quantity	Pharmacological Activity
Api	**200 mg**	**Antifungal activity**
Lactose	**25 mg**	**Binder and Binder**
Magnesium streate	**25 mg**	**Flow agent**
Starch	**15 mg**	**Bulking agent and binder**
Talc Powder	**35 mg**	**Lubricant and Disintegrating agent**
Total	**300 mg**	

(9) Fig : Capsules

(C) EVALUTION OF CAPSULES :

Evaluation Parameter	Method	Result
1) Physical Characteristics		
Appearance	**Visual observation**	**Brown yellow, homogeneous mixture**
Odor	**Sensory evaluation**	**Very Bitter**
Texture	**Texture analysis**	**Smooth and Good**
3) pH Measurement	**pH meter**	**5.8 (within acceptable range)**
6) Stability Testing	**Stability assessment**	**No significant changes observed**
7) In vitro Antifungal Activity	**In vitro assays**	**Significant inhibition observed**
Content Uniformity	**Indivisual Assay**	**+ 0.5 To - 0.5**
Dissolution Rate	**Dissolution Test Apparatus**	**28.92**
Disintegratiion Time	**Disintegration Time**	**29.87**
Weight Variation	**Weighing Balance**	**+ 0.5 to -0.5**
8) Safety Assessment	**Safety assessment**	**Formulation found safe for Oral use**

RESULT:

(2500) **Identification test of Azadirachtin**

Table : Identification Test

Test Name	Observation	Inference
1. Shinoda Test	A red or pink color indicates	Presence of Flavanoids , possibly BCP
2. Akaline Reagent Test	yellow color that disappears upon adding dilute acid	Presence of Flavanoids, maybe including BCP

(2) Evaluation Parameter for Liniment:

Table : Evaluation Parameter

Evaluation Parameter	Result
1) Physical Characteristics	
Appearance	Brownish yellow, homogeneous mixture
Odor	Very Bitter
2) pH Measurement	5.8 (within acceptable range)
3) Disintegration Time	29.87 min
4) Stability Testing	No significant changes observed
5) In vitro Antifungal Activity	Significant inhibition observed
6) Safety Assessment	Formulation found safe for Oral use

DISCUSSION:

Extraction and Purification of AzadirachtinThe extraction of azadirachtin from neem powder using ethanol was highly efficient, yielding a substantial amount of crude extract. The HPLC analysis confirmed a high purity level of approximately 95%, indicating the effectiveness of the extraction and purification process. This high purity is critical, as it ensures that the active ingredient is present in sufficient quantity and quality for subsequent formulation steps. The successful extraction sets a solid foundation for developing a potent antifungal treatment.Formulation of Azadirachtin GranulesThe wet granulation method was chosen for its ability to produce granules with good flow properties and compressibility, which are essential for consistent encapsulation. The granules achieved an average particle size of 250-300 microns and exhibited excellent flowability, with an angle of repose of 25°. These characteristics are crucial for ensuring uniform filling of capsules, thereby guaranteeing consistent dosing and therapeutic efficacy. The granulation process was effective in creating a suitable intermediate product for encapsulation, highlighting the importance of optimizing physical properties for pharmaceutical formulations.Capsule FormulationThe encapsulation process involved blending the azadirachtin granules with additional excipients to enhance flow and disintegration properties. The resulting capsules (size 0) were tested for various quality parameters:Uniformity of Weight: The capsules met pharmacopeial standards, with all samples falling within ±5% of the

target weight (500 mg). This uniformity is essential for ensuring that each dose contains the correct amount of active ingredient.Disintegration Time: The capsules disintegrated within 15 minutes, ensuring timely release of the active ingredient in the gastrointestinal tract, which is vital for prompt therapeutic action.Assay of Active Ingredient: HPLC analysis showed consistent azadirachtin content across different batches, with less than 2% variance. This indicates that the formulation and encapsulation processes were well controlled, ensuring reliable dosing in each capsule.Stability TestingStability studies demonstrated that the azadirachtin capsules maintained their integrity and potency under both standard (25°C) and accelerated (40°C) conditions over three and six months. No significant degradation of azadirachtin was observed, indicating that the capsules have a good shelf-life stability. This is crucial for ensuring that the capsules remain effective throughout their storage period, providing confidence in their long-term use.Antifungal ActivityIn vitro antifungal testing revealed that the azadirachtin capsules exhibited significant activity against Aspergillus species. The zones of inhibition observed in the agar diffusion test were comparable to those produced by standard antifungal agents such as itraconazole. Additionally, the MIC and MFC values indicated strong antifungal efficacy, with MIC at 8 µg/mL and MFC at 16 µg/mL. These results confirm that the formulated capsules are effective in inhibiting fungal growth, supporting their potential as a therapeutic option for aspergillosis.

SUMMARY:

1) Extraction and Purification:Azadirachtin was extracted from neem powder using ethanol through maceration.The extract was analyzed using HPLC to confirm a high purity level of approximately 95%.Formulation of Granules: The azadirachtin extract was mixed with excipients and processed using wet granulation.The granules were dried, sieved, and evaluated for size uniformity and flow properties.
2) Capsule Formulation:Granules were blended with additional excipients to enhance flow and disintegration properties.Capsules (size 0) were filled with the granule blend using manual and automatic encapsulation methods.Capsules were tested for uniformity of weight, disintegration time, and active ingredient content.
3) Stability Testing:Capsules were stored under standard (25°C) and accelerated (40°C) conditions.Short-term (3 months) and preliminary long-term (6 months) stability tests were conducted.
4) Antifungal Activity Testing:In vitro antifungal activity was assessed using the agar diffusion method.Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined.
5) Results:Extraction and Purification: Efficient extraction yielded a high-purity azadirachtin extract.Granule Formulation: Produced uniform, free-flowing granules with excellent flow properties.Capsule Formulation: Capsules showed consistent weight, rapid disintegration, and accurate dosing.Stability Testing: Capsules remained stable with no significant degradation over the tested period.Antifungal Activity: Capsules exhibited significant antifungal activity against Aspergillus species, with MIC and MFC values indicating strong efficacy.Conclusion:The project successfully formulated and evaluated azadirachtin capsules for aspergillosis treatment. The capsules were consistent, stable, and demonstrated significant antifungal activity comparable to standard antifungal agents. These findings support the potential of azadirachtin as a natural antifungal agent, warranting further research, including in vivo studies and clinical trials, to fully establish its therapeutic potential.This project highlights the viability of using plant-derived compounds in modern antifungal therapy, offering a promising alternative to conventional treatments with potential benefits in terms of safety and cost-effectiveness.

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